# Determination of reference range values indicative of glucose metabolism and insulin resistance by use of glucose clamp techniques in horses and ponies

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**Objectives**—To acquire reference range values indicative of glucose metabolism by use of the hyperglycemic clamp technique in healthy horses and evaluate the usefulness of the euglycemic hyperinsulinemic clamp technique in healthy horses and ponies.

**Animals**—5 Dutch Warmblood horses and 4 Shetland ponies.

**Procedure**—The hyperglycemic clamp technique was used for quantification of the sensitivity of beta cells to exogenous glucose infusion in horses. The euglycemic hyperinsulinemic clamp technique was used to determine the sensitivity and responsiveness of tissues to exogenous insulin in horses and ponies.

**Results**—During the hyperglycemic clamp technique, the mean amount of glucose metabolized (M) in horses was 0.011 ± 0.0045 mmol/kg·min$^{-1}$ (95% confidence interval [CI], 0.0018 to 0.020 mmol/kg·min$^{-1}$; range, 0.000035 to 0.021 mmol/kg·min$^{-1}$) and the mean M value-to-plasma insulin concentration (I) ratio (ie, mmol of glucose/kg·min$^{-1}$ per pmol of insulin/L × 100) was 0.017 ± 0.016 (95% CI, –0.014 to 0.049; range, 0.000025 to 0.055). During the euglycemic hyperinsulinemic clamp technique, the mean M value was 0.014 ± 0.0055 mmol/kg·min$^{-1}$ (95% CI, 0.0026 to 0.025 mmol/kg·min$^{-1}$; range, 0.0042 to 0.023 mmol/kg·min$^{-1}$) in horses and 0.0073 ± 0.0020 mmol/kg·min$^{-1}$ (95% CI, 0.0034 to 0.011 mmol/kg·min$^{-1}$; range, 0.0049 to 0.011 mmol/kg·min$^{-1}$) in ponies. The M value was significantly lower in ponies than in horses, whereas the M:I ratios were not significantly different between horses and ponies.

**Conclusion and Clinical Relevance**—Glucose clamp techniques offer good methods to investigate glucose metabolism in horses and ponies. A higher degree of insulin resistance was found in ponies, compared with Dutch Warmblood horses. (*Am J Vet Res* 2003;64: 1260–1264)

Insulin is synthesized and secreted by beta cells of the islets of Langerhans in the pancreas and regulates glucose utilization and production. Liver, muscle, and adipose tissue are target tissues. Insulin resistance is defined as a condition in which normal concentrations of insulin produce a subnormal physiologic response.[1] Insulin resistance is found in physiologic (eg, starvation or gestation) or pathologic (eg, diabetes mellitus, obesity, uremia, or cortisol or growth hormone excess) conditions.[2] Two important endocrine diseases in horses, which are accompanied by supposed insulin resistance, are hyperadrenocorticism and hyperlipemia. Mobilization of fatty acids appears to be poorly regulated in ponies because of an innate peripheral insensitivity to insulin.[3] Horses with hyperadrenocorticism generally have a high baseline plasma insulin concentration accompanied by a high resistance to insulin.[4]

To study insulin resistance, a glucose clamp technique was developed by Andres et al[5] in 1966. The glucose clamp technique allows direct assessment of insulin sensitivity in vivo,[6] in which a constant glucose-insulin negative feedback mechanism is present. An increase in plasma glucose concentration stimulates beta cell release of insulin; the resultant increase in plasma insulin concentration stimulates cellular uptake of glucose, and the plasma glucose concentration decreases. These processes occur simultaneously, and neither plasma glucose nor insulin concentrations are held constant. Use of the glucose clamp technique places the plasma glucose concentration under the investigator's control and thus breaks the glucose-insulin negative feedback loop.[7] Two types of glucose clamp techniques exist, the hyperglycemic clamp technique and the euglycemic hyperinsulinemic clamp technique.

The hyperglycemic clamp technique allows quantification of the sensitivity of beta cells to glucose. The hyperglycemic clamp technique is used to increase the plasma glucose concentration acutely to a fixed hyperglycemic plateau and maintain it at that concentration for approximately 2 hours, thereby suppressing basal hepatic glucose production. Because the plasma glucose concentration is held constant, the glucose infusion rate is an index of glucose metabolism.[7]

The euglycemic hyperinsulinemic clamp technique allows quantification of the sensitivity of tissues to insulin. By maintaining euglycemia during insulin infusion, an increase in endogenous insulin secretion is avoided. The principle of the euglycemic hyperinsulinemic clamp technique is that the rate of glucose infusion required to maintain euglycemia equals the quantity of glucose taken up by all tissues in response to exogenous insulin administration, provided that endogenous glucose entry rate remains constant. The glucose infusion rate necessary to maintain euglycemia during the insulin-infusion period increases gradually. For this reason, only glucose infusion rates during a plateau period are used to quantitate the actions of insulin on glucose uptake by tissues.[7]

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In humans, a variety of studies[6,7] on the use of glucose clamp techniques to assess insulin responsiveness and sensitivity have been published. Studies on the use of the glucose clamp technique in animals has involved mostly ruminants (ie, sheep[8-10] and cows[a]). Similar studies on horses are limited. To our knowledge, the use of the hyperglycemic clamp technique in horses has not been investigated, and the use of the euglycemic hyperinsulinemic clamp technique in horses and ponies has only been investigated by Elmahdi.[b] The purposes of the study reported here were to acquire reference range values indicative of glucose metabolism by use of the hyperglycemic clamp technique in healthy horses and evaluate the usefulness of the euglycemic hyperinsulinemic clamp technique in healthy horses and ponies.

## Materials and Methods

**Animals**—The Committee on Animal Welfare of the Faculty of Veterinary Medicine, Utrecht University, approved this study. Five Dutch Warmblood horses (3 mares and 2 geldings) and 4 Shetland ponies (geldings) were included in the study. The age of the Dutch Warmblood horses ranged from 3 to 16 years (mean ± SD, 9.8 ± 5.1 years) and body weight of the Dutch Warmblood horses ranged from 489 to 588 kg (549 ± 38.0 kg). The age of the Shetland ponies ranged from 2 to 8 years (4.5 ± 2.6 years old), and body weight of the Shetland ponies ranged from 132 to 156 kg (140 ± 11.0 kg). Food was withheld from horses and ponies for 15 hours prior to and during the experiment. Horses were included in the investigation of the hyperglycemic and euglycemic hyperinsulinemic clamp techniques in a 2-factor crossover design with an interval of 7 days, whereas the Shetland ponies were included in the investigation of the euglycemic hyperinsulinemic clamp technique only.

**Hyperglycemic clamp technique**—On the day of the experiment at approximately 10:30 AM, polyvinylchloride catheters were inserted into both jugular veins. One of the catheters was used for the infusion of glucose, and the other catheter was used for obtaining blood samples at 10-minute intervals according to the protocol by Bergman et al.[6]

Prior to the experiment, horses were weighed to calculate the priming dose of glucose, which was used to rapidly induce a plasma glucose concentration of > 10.8 mmol/L. The priming dose that was calculated was similar to 50% of that used in clinically normal humans (3.35 µmol/kg).[7] At time zero (t = 0), a blood sample was taken for determination of basal plasma glucose concentration. Hereafter, the body weight-dependent priming dose, which varied from 120 to 140 mL (mean ± SD, 132 ± 8.4 mL) of glucose as a 50% solution, was given IV within 2 minutes.

Every 10 minutes during the experiment, 2 blood samples were collected for determination of glucose and insulin concentrations. For determination of glucose concentration, blood was collected into heparinized syringes. Within 2 minutes, the glucose concentration of the heparinized blood sample was determined by use of an automated analyzer,[c] which measured the potassium concentration simultaneously. To check the measurements of the automated analyzer during the experiment, 3 additional blood samples (collected into tubes that contained sodium fluoride and potassium EDTA) were sent to the laboratory for plasma glucose concentration determination via another automated analyzer.[d] When the concentration of glucose was < 10 mmol/L, the glucose infusion, by use of a peristaltic syringe pump,[e] was started at a mean rate of 122 ± 9.8 mL/h. Glucose infusion rate was adjusted when the preceding blood glucose concentration differed from that of 10.8 to 12.5 mmol/L. The glucose infusion was stopped after maintaining a steady state for > 40 minutes. As many urine samples as possible were collected for the determination of urine glucose concentration. The IV catheters were removed, and horses were brought back to their stalls.

For determination of insulin concentration, blood was collected into tubes that contain lithium heparin. Plasma insulin concentration was determined in 4 samples obtained within 10-minute intervals during the hyperglycemic steady state. These blood samples were centrifuged for 5 minutes at 6,000 × g.[f] Plasma was separated and stored at –20°C until analysis of insulin was performed. Plasma insulin concentration was measured by use of a radioimmunoassay kit[g] validated for use in horses.[11]

**Euglycemic hyperinsulinemic clamp technique**—The experimental procedure used was adapted from that described by Elmahdi.[b] On the day of the experiment at approximately 10:30 AM, polyvinylchloride catheters were inserted into both jugular veins. One of the catheters was used for the infusion of glucose as a 50% solution and insulin,[h] whereas the other catheter was used for obtaining blood samples. Two peristaltic pumps were used; 1 for the infusion of insulin[i] and the other for the infusion of glucose.[e] Separate infusion lines between the pumps and catheter were used for insulin and glucose infusion. Prior to the experiment, horses and ponies were weighed to calculate the precise amount of insulin necessary to induce a plasma insulin concentration indicative of a hyperinsulinemic condition. A control blood sample (t = 0) was taken for determination of basal glucose concentration, after which a priming dose of insulin (323 µmol/kg), dissolved in 50 mL of sodium chloride as a 0.9% solution, was given IV within 10 minutes. Directly following administration of the priming dose, the insulin infusion pump was started with a constant infusion rate of 43 µmol/kg·min$^{-1}$. Glucose infusion was started simultaneously with a mean infusion rate of 8.6 µmol/kg·min$^{-1}$ in horses and 7.2 µmol/kg·min$^{-1}$ in ponies. During the insulin and glucose infusions, blood samples were obtained every 10 minutes.

The blood glucose concentration was assayed within 2 minutes by use of an automated analyzer,[c] which measured the potassium concentration simultaneously. Glucose infusion rate was adjusted as soon as the preceding blood glucose concentration differed from a concentration of 3.9 to 5.6 mmol/L. To check the measurements of the automated analyzer[c] during the experiment, 3 additional blood samples (collected into tubes that contained sodium fluoride and potassium EDTA) were sent to the laboratory for determination of plasma glucose concentration via another automated analyser.[d]

Plasma insulin concentration was determined in 4 blood samples that were collected in tubes that contain lithium heparin at 10-minute intervals during the euglycemic hyperinsulinemic condition. These blood samples were centrifuged for 5 minutes at 6,000 × g.[f] Plasma was separated and stored at –20°C until insulin concentrations were measured by use of a radioimmunoassay kit[g] validated for use in horses.[11] After maintaining a steady state for > 40 minutes, the insulin and glucose infusions were stopped. To prevent hypoglycemia following the experimental procedure, horses and ponies were first given 200 to 300 mL of glucose as a 50% solution IV. After removal of the catheters, horses and ponies also received carbohydrates in the form of 1 kg of concentrate feed along with roughage.

**Calculations**—During steady state, the **amount of glucose infused (INF)** must equal the **amount of glucose metabolized (M)**, provided that endogenous glucose production is

completely suppressed by hyperinsulinemia or hyperglycemia.[7] The INF (50% solution; mmol/kg·min$^{-1}$) was calculated by use of the following equation:

$$INF = \frac{mL/h \times 8.333}{BW \times 180}$$

where BW stands for body weight in kilograms.

In practice, 2 correction factors are necessary. With reference to the hyperglycemic clamp technique, a small correction for the **amount of glucose loss in urine (UC)** has to be made. The duration of glucose infusion was approximately 2 hours. To calculate the amount of glucose loss via urine over this period, the urine glucose concentration was determined in 3 urine samples collected immediately after ending the hyperglycemic clamp,[7] resulting in a mean urinary glucose concentration of 23.5 mmol/L. To calculate the UC, 24-hour urine production in the horse was estimated as approximately 10 L.[12] The UC (mmol/kg·min$^{-1}$) was calculated by use of the following equation:

$$UC = \frac{29.37}{BW \times 180}$$

In the hyperglycemic and euglycemic hyperinsulinemic clamp techniques, the plasma glucose concentration is not maintained perfectly constant, and a correction, therefore, must be made. The **space correction (SC)** adjusts for glucose that has been added or removed. The formula for SC is determined on the basis of human values, with the assumption that 5 mg or 0.028 mmol of glucose is removed from each deciliter of glucose space in a 20-minute period.[7] In the current experiment, the M is computed for a period of 10 minutes. The plasma glucose concentrations in mmol/L at the beginning ($G_1$) and end ($G_2$) of the 10 minutes are considered. The glucose space in L is given by 0.19 × BW. The SC was calculated by use of the following equation:

$$SC = \frac{(G_2 - G_1) \times (0.19 \times BW)}{10 \times BW}$$

The equation reduces to the following:

$$SC = (G_2 - G_1) \times 0.019$$

The M (mmol/kg·min$^{-1}$) was calculated by use of the following equation:

$$M = INF - UC - SC$$

In the hyperglycemic clamp technique, the **plasma insulin concentration (I**; pmol/L) during the steady state is a measure of the beta cell response to glucose. The M:I ratio reflects the quantity of glucose metabolized per unit of insulin in plasma and, as a result, is a reasonable index of the sensitivity of tissues to insulin.[7] To compare our results with those from DeFronzo et al,[7] the M:I ratio was multiplied by 100. DeFronzo et al[7] only determined the M:I ratio during use of the hyperglycemic clamp technique. During hyperglycemia, glucose uptake is enhanced, and as a consequence, determination of M may overestimate the amount of insulin-mediated glucose uptake. This is corrected by computing the M:I ratio. In this study, M:I ratios during use of the euglycemic hyperinsulinemic clamp were also calculated.

**Assays and data analysis**—Plasma insulin and glucose concentrations were determined as previously described.[11] Results are expressed as mean (± SD) values. Differences in M values and M:I ratios between horses and ponies during use of the euglycemic hyperinsulinemic clamp technique were analyzed by means of a linear mixed-effects model with 1-step autoregressive process. The strength of linear relationships was assessed by obtaining the correlation coefficient (r) and testing whether it was different from zero by use of the Pearson test (2-tailed). The change in plasma potassium concentration was analyzed by use of a paired *t* test. Values of $P < 0.05$ were considered significant.

## Results

After a 15-hour period of withholding food, the mean basal plasma glucose concentration in horses was 5.4 ± 0.34 mmol/L and in ponies was 3.8 ± 0.13 mmol/L. The correlation coefficient between glucose concentrations measured in heparinized blood samples[c] versus plasma obtained from blood collected into tubes that contained sodium fluoride[d] was high (r = 0.988; n = 30; $P < 0.001$). Mean heparinized blood potassium concentrations were within reference range values immediately following the end of the glucose clamp techniques (reference range values, 3.0 to 5.9 mmol/L). Mean heparinized blood potassium concentration, however, decreased significantly ($P = 0.001$) in horses only following the end of the euglycemic hyperinsulinemic clamp technique (from 3.7 ± 0.27 to 3.0 ± 0.23 mmol/L).

**Hyperglycemic clamp technique**—The hyperglycemic clamp technique was applied to 5 Dutch Warmblood horses in which the mean time to reach a steady state plasma glucose concentration that was indicative of hyperglycemia (ie, > 10.8 mmol of glucose/L) was 156 ± 73 minutes. During steady state, mean plasma glucose concentration was 11.2 ± 0.41 mmol/L (**Fig 1**), and the corresponding mean plasma insulin concentration was 117.0 ± 70.3 pmol/L. During steady state plasma glucose concentrations, the glucose infusion rate was corrected for UC and SC (SC was -0.137 ± 0.670 mmol/kg·min$^{-1}$, as determined on the basis of 15 observations in 5 horses) and equaled the M value. Urinalysis results revealed urine glucose concentrations of 4.0, 18.6, and 47.8 mmol/L. The mean M value was 0.011 ± 0.0045 mmol/kg·min$^{-1}$ (95% **confidence interval [CI]**, 0.0018 to 0.020 mmol/kg·min$^{-1}$; range, 0.000035 to 0.021 mmol/kg·min$^{-1}$, as deter-

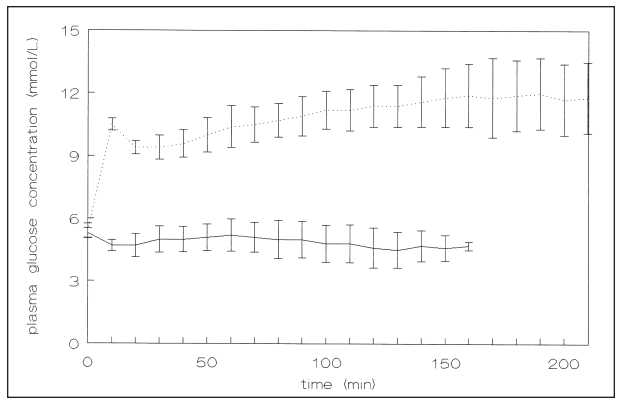


Figure 1—Mean (± SD) plasma glucose concentration during the hyperglycemic clamp technique (n = 5; upper dotted line) and the euglycemic hyperinsulinemic clamp technique (n = 4; lower solid line) in Dutch Warmblood horses.

mined on the basis of 15 values). The mean M:I ratio (ie, mmol of glucose/kg·min$^{-1}$ per pmol of insulin/L × 100) was 0.017 ± 0.016 (95% CI, –0.014 to 0.049; range, 0.000025 to 0.055).

**Euglycemic hyperinsulinemic clamp technique**—The euglycemic hyperinsulinemic clamp technique began with the administration of the priming dose of insulin (323 µmol/kg) that was given within 10 minutes after obtaining a control blood sample. After administration of the priming dose, mean plasma glucose concentration decreased significantly from baseline values in horses ($P$ = 0.012) by 0.76 ± 0.39 mmol/L and in ponies ($P$ = 0.037) by 0.23 ± 0.13 mmol/L. Steady state plasma glucose and insulin concentrations that were indicative of a euglycemic hyperinsulinemic condition were reached in mean times of 104 ± 52.7 minutes in horses and 57.5 ± 22.2 minutes in ponies. The mean plasma insulin concentration, as determined on the basis of 4 measurements per animal during the hyperinsulinemic condition, was 4,391 ± 1,988 pmol/L in horses and 1,995 ± 660 pmol/L in ponies. The mean plasma glucose concentration during hyperinsulinemia was 4.5 ± 0.31 mmol/L in horses and 4.0 ± 0.42 mmol/L in ponies, both of which were within the euglycemic range (SC for horses = -0.388 ± 1.006 mmol/kg·min$^{-1}$; SC for ponies = -0.057 ± 0.365 mmol/kg·min$^{-1}$). Urinalysis results for Dutch Warmblood horses revealed urine glucose concentrations of 0.3, 0.8, 1.2, and 7.1 mmol/L; urinalysis results for ponies revealed urine glucose concentrations of 0.13 and 0.5 mmol/L. These urine glucose concentrations justified the small correction for UC used only during the hyperglycemic clamp technique. The M value in the euglycemic hyperinsulinemic clamp technique is a measure of the sensitivity of tissues to exogenous insulin and had a mean value in horses of 0.014 ± 0.0055 mmol/kg·min$^{-1}$ (95% CI, 0.0026 to 0.025 mmol/kg·min$^{-1}$; range, 0.0042 to 0.023 mmol/kg·min$^{-1}$) and in ponies of 0.0073 ± 0.0020 mmol/kg·min$^{-1}$ (95% CI, 0.0034 to 0.011 mmol/kg·min$^{-1}$; range, 0.0049 to 0.011 mmol/kg·min$^{-1}$). The M value in ponies was significantly lower than in horses. The mean M:I ratio (× 100) in horses of 0.00038 ± 0.00020 (95% CI, –0.000027 to 0.00079; range, 0.00007 to 0.00082) was not significantly different from the mean M:I ratio (× 100) in ponies of 0.00039 ± 0.00010 (95% CI, 0.00019 to 0.00060; range, 0.00027 to 0.00057).

In 1 of the 5 horses, the euglycemic hyperinsulinemic clamp technique was repeated to evaluate the reproducibility of results. On day 0 (ie, the first time the euglycemic hyperinsulinemic clamp technique was performed), a priming insulin dose of 646 µmol/kg was administered as an IV bolus, and the resulting M value was 0.013 ± 0.007 mmol/kg·min$^{-1}$, and the corresponding M:I ratio (× 100) was 0.00037 ± 0.00019. On days 13 and 17 (ie, the second and third time the euglycemic hyperinsulinemic clamp technique was performed), a priming insulin dose of 323 µmol/kg was administered, and the resulting M values were 0.016 ± 0.0029 and 0.016 ± 0.0039 mmol/kg·min$^{-1}$, respectively, and the corresponding M:I ratios (× 100) were 0.00057 ± 0.00007 and 0.00067 ± 0.00012, respectively. In terms of the sensitivity of tissues to insulin, the relationship in 5 horses between M:I ratios, as determined by use of the hyperglycemic clamp technique, and the M values, as determined by use of the euglycemic hyperinsulinemic clamp (M), were not significant (r = –0.258; $P$ = 0.675). However, the relationship between the M-values as determined by use of both clamps was significant (r = 0.642; $P$ = 0.010).

## Discussion

The euglycemic hyperinsulinemic clamp technique provides a method for quantification of the sensitivity of tissue to insulin. Our study was conducted on the basis of studies by Elmahdi[b] and Defronzo et al,[7] but some adjustments were made. DeFronzo et al[7] described a period of 4 minutes between the start of insulin infusion and glucose infusion. Because administration of the priming insulin dose usually results in hypoglycemia, we preferred to start the glucose infusion simultaneously with the insulin infusion. Elmahdi[b] used porcine insulin, whereas in our study, human insulin was used, because no porcine insulin that could be administered IV was available. Human insulin differs from the structure of equine insulin by 2 amino acids, whereas porcine insulin only differs from the structure of equine insulin by 1 amino acid and is therefore expected to be more potent in horses and ponies. Elmahdi[b] used a sterile glucose solution of 20%. In our study, a sterile glucose solution of 50% was administered, because this resulted in a lower total fluid infusion. Elmahdi[b] used insulin infusate that contained potassium chloride as well to avoid insulin-induced hypokalemia. In our study, no potassium was added, but the blood potassium concentration was monitored every 10 minutes during insulin infusion. Mean heparinized blood potassium concentration following the end of both glucose clamp techniques did not decrease below the lower limit of the reference range values. Elmahdi[b] used a priming insulin dose of 646 µmol/kg, followed by an insulin infusion rate of 43 µmol/kg·min$^{-1}$. By using this priming dose, we found that initially horses became hypoglycemic, and glucose concentrations could not be corrected by increasing the glucose infusion rate as a result of a limited capacity of our syringe pump. Therefore, the priming dose of insulin used in our study was adjusted to 323 µmol/kg. Supraphysiologic plasma insulin concentrations are necessary to produce a maximal response of glucose uptake by tissues and inhibit endogenous hepatic glucose production. In humans and rats, the maximal response of glucose uptake by tissues during the use of the euglycemic hyperinsulinemic clamp technique was achieved at insulin plasma concentrations that ranged from 1,435 to 5,023 pmol/L.[13,14] In our study as a comparison, mean plasma insulin concentrations were 4,391 ± 1,988 pmol/L in horses and 1,995 ± 660 pmol/L in ponies. The liver of healthy humans is sensitive to small incremental increases in plasma insulin concentration. A 718 pmol/L incremental increase in the plasma insulin concentration will decrease hepatic glucose production to < 10 to 15% of basal concentrations in humans.[15]

In our study, glucose metabolism by horses and ponies during the euglycemic hyperinsulinemic clamp was not similar. The basal plasma glucose concentra-

tion of the ponies (3.8 ± 0.13 mmol/L) after withholding food was much lower than that of horses (5.4 ± 0.34 mmol/L). Elmahdi[b] found basal plasma glucose concentrations of 3.86 ± 0.1 mmol/L in ponies and 3.89 ± 0.2 mmol/L in horses. Horses and ponies also differed in our study in the way that they reacted to the priming insulin dose of 323 µmol/kg. Plasma glucose concentration decreased 3 times as much in horses as in ponies. The M value during the insulin bolus as a consequence was lower in ponies and provided us with a first indication of a higher insulin resistance in ponies than in horses. The M value during the euglycemic hyperinsulinemic clamp technique is a measure of the sensitivity of tissues to exogenous insulin and in our study had a mean value of 0.014 ± 0.0055 mmol/kg·min$^{-1}$ in horses and 0.0073 ± 0.0020 mmol/kg·min$^{-1}$ in ponies. Elmahdi[b] found M values of 0.0086 ± 0.0011 mmol/kg·min$^{-1}$ in horses and 0.0072 ± 0.00068 mmol/kg·min$^{-1}$ in ponies. Elmahdi[b], however, used crossbred rather than purebred horses. In both studies, however, ponies were less sensitive to exogenous insulin than horses. These findings are in agreement with the knowledge that ponies have an innate insulin resistance.[3]

The hyperglycemic clamp technique allows for determination of the sensitivity of beta cells of the pancreas to glucose, the determination of the total M value in tissues, and the determination of the sensitivity of tissues to endogenous insulin.[7] Because no values for horses are available, our results were compared with those found in humans. The mean (± SE) M value in humans was reported as 0.045 ± 0.0038 mmol/kg·min$^{-1}$, and the corresponding mean M:I ratio was 0.010 ± 0.00091.[6] The results of our study indicated that the M value of horses is lower than in humans, probably as a result of the lower insulin secretion of beta cells during hyperglycemia in horses. Mean plasma insulin concentration in horses of our study was 117.0 ± 70.3 pmol/L, compared with a mean (± SE) value of 445 ± 22 pmol/L (SE) in humans. The sensitivity of tissue to endogenous insulin (M:I ratio) was higher in horses than in humans. In comparison, the relationship between the sensitivity of tissues to insulin as determined by use of the hyperglycemic clamp technique (M:I ratio) and the euglycemic hyperinsulinemic clamp technique (M value) in 11 humans was high ($r = 0.816$; $P < 0.01$).[7]

In our study, the mean M:I ratio (× 100) in horses was 0.00038 ± 0.00020 and in ponies was 0.00039 ± 0.00010. DeFronzo et al[7] did not determine the M:I ratios during the use of the euglycemic hyperinsulinemic clamp technique. The plasma insulin concentration during the euglycemic hyperinsulinemic clamp technique is dependent on the infusion of exogenous insulin. During hyperinsulinemia, hepatic glucose production is suppressed, and glucose metabolism is maximized. A further increase in the insulin infusion rate does not increase the amount of metabolized glucose; on the other hand, it does decrease the M:I ratio. For investigation of insulin resistance, the euglycemic hyperinsulinemic clamp technique provides a more reliable measure of the sensitivity of tissue to insulin, because all of the infused (species specific) insulin is known to be biologically active, whereas a small percentage of the insulin secreted during the hyperglycemic clamp technique is proinsulin, which is biologically less potent than insulin.[7] Results of our study indicate that both types of glucose clamp techniques can be used in horses and ponies and may help to increase our insight into the pathogenesis of diseases like hyperlipemia and hyperadrenocorticism, both of which are characterized by a disturbance in glucose metabolism.

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[c]ABL-605 radiometer Copenhagen, Westlake, Ohio.

[d]Beckman, Mijdrecht, the Netherlands.

[e]Terumo model STC-521, Louven, Belgium.

[f]Hettich zentrifugen, Tuttlingen, Germany.

[g]Diagnostic Products Corp, Los Angeles, Calif.

[h]Actrapid recombinant human insulin (100 U/mL), Novo Nordisk A/S, Bagsvaerd, Danmark.

[i]Vickers Medical Treonic IP4 syringe pump, Basingstoke, Hampshire, UK.

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